Original Article

MOLECULAR METABOLISM

Disentangling the effects of obesity and high-fat diet on glucose homeostasis using a photoperiod induced obesity model implicates ectopic fat deposition as a key factor

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ABSTRACT

Objective: Obesity in laboratory rodents is generally induced by feeding them a high fat diet (HFD). This model does not permit separation of the impact of the HFD from the resultant obesity on metabolic defects such as impaired glucose homeostasis. In Brandt's voles we have previously shown that exposure to long photoperiod (LD: 16L: 8D) induces obesity even when they are fed a low fat diet. We show here that these voles are largely resistant to HFD. This model therefore permits some separation of the effects of HFD and obesity on glucose homeostasis. The objective was therefore to use this model to assess if glucose homeostasis is more related to diet or obesity

Methods: Male voles, which were 35 days old and born in LD, were exposed to SD and a low fat diet for 70 days. We then randomly separated the animals into 4 groups for another 63 days: SL (short day and low fat diet: $n = 19$) group; SH (short day and high-fat diet, $n = 20$) group; LL (long day and low-fat diet, $n = 20$) group; LH (long day and high-fat diet, $n = 18$) group. Glucose tolerance tests (GTT) were performed after treatment for 56 days, and body compositions of the voles were quantified at the end by dissection.

Results: Consistent with our previous work LD voles were more obese than SD voles. Although total body weight was independent of dietary fat content, HFD did have an effect on fat storage. Photoperiod induced obesity had no effect on glucose homeostasis, and the fat content in both the liver and muscle. In contrast, HFD induced adiposity was linked with elevated fat deposition in muscle (but not in liver) and led to impaired glucose tolerance.

Conclusions: The contrasting effects of diet and photoperiod were consistent with the predictions of the 'lipotoxicity hypothesis'. This may contribute to our understanding of why some human individuals are able to be obese yet remain metabolically healthy.

Keywords Brandt's vole; Photoperiod-induced obesity; High-fat diet; Ectopic fat deposition; Glucose tolerance

1. INTRODUCTION

The world is currently in the middle of a pandemic of obesity, and the spiraling increase in obesity rates has caused a host of related metabolic complications such as type 2 diabetes [1–5]. Although obesity is a primary risk factor for type 2 diabetes, and there is generally a link between the degree of adiposity and the level of insulin resistance, the association is not inevitable [2,3,6–8]. A population of people with obesity appears able to remain healthy and avoid the negative implications it often normally entails. This population has been generally termed as the 'healthy obese' [6,8]. Understanding why some people are able to become obese without the metabolic complications is important because this may point a way towards novel therapeutic options that will help reduce the translation of obesity into diabetes. Given the general failure of most attempts to reverse the spread of obesity, stemming the translation from obesity to diabetes may be a more effective option. To achieve an understanding of how obesity and diabetes are linked mechanistically has been, and will

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continue to be, a key focus of research effort. Progress however is hindered by the availability of good animal models for the 'healthy obesity' phenomenon.

The very existence of healthy obesity suggests the problem is not obesity per se but the dietary regime that led to the obesity, or how ingested excess energy is handled [6,9–11]. Rodent animal models of obesity (and obesity induced glucose intolerance) have largely involved feeding mice or rats diets with high fat contents: known as the high fat diet (HFD) feeding, or diet induced obesity (DIO) model [12–14]. One issue with this model is that it is not possible to separate the effects of obesity from the effects of the diet that is used to induce the obesity, since it is not possible to get normal mice to overconsume and become obese on control low fat diets (LFD).

Many wild animals deposit large adipose tissue stores in advance of some anticipated period of food shortage, or for a period of high demand such as trans-global migration [15–18]. Moreover, many other non-hibernating animals like small rodents (such as voles, hamsters and lemmings) go through annual cycles of fattening that are not obviously linked to periods of migration, or low food supply [19–22]. Voles are small non-hibernating rodents that are common throughout the palearctic and nearctic regions. We have shown that voles have an annual cycle of body weight change that reflects large changes in their levels of adiposity [23,24] and that this state can be induced by manipulations of photoperiod, independent of the diet [25–30]. This photoperiod induction of adiposity, provides us with a powerful model where, simply by switching the light regime in the room where the animals are housed, we can turn a lean vole into a vole with obesity within the space of about 5 weeks without any change in the diet. We have additionally shown that bank voles (*Myodes glareolus*) on SD do not develop obesity when placed on a HFD [30–33]. Similar resistance in Brandt's voles under SD conditions has been reported [29,30,34]. Although in that case there was some increased deposition of fat under HFD in long days, this contrasting effect of photoperiod and HFD on adiposity in voles opens up the possibility to disentangle the effects of obesity from the effects of HFD by combining the photoperiod induced obesity model with dietary manipulations.

In the present study we exposed Brandt's voles (*Myodes brandtii*) to either long or short photoperiods. and under each photoperiod treatment exposed them also to either a high or low-fat diet. For the HFD we used a diet with 26% calories from fat. This is because voles refused to eat diets with very high fat contents (>40%), and 26% is more in line with recommendations to match human obesogenic diets [35]. We aimed to confirm the stability of the photoperiod-induced obese model in Brandt's vole that we have already established [29] and to characterize the impact of HFD feeding in this model. Second, we explored what the consequences of the photoperiod-induced obese state and high-fat diet were for glucose tolerance. Finally, we explored the extent to which fat is deposited in the liver and skeletal muscle under these different treatments to establish if modulations of glucose homeostasis were consistent with alterations in the levels of ectopic fat deposition.

2. MATERIAL AND METHODS

2.1. Animals and experimental design

2.1.1. Animals

All animal procedures were carried out in agreement with the Animal Care and Use Committee, Chinese Academy of Sciences. Brandt's voles from our laboratory colony were live-trapped in Inner Mongolia and maintained at 22 ± 2 °C on a 16h: 8h light: dark cycle (lights on at 04:00). Animals were individually housed in plastic cages (30 × 15 × 20 cm) with sawdust bedding, food (standard rabbit pellet chow, Beijing HFK Bioscience Co.) and water provided *ad libitum*.

2.1.2. Experimental protocols

Male Brandt's voles ($n = 76$) from our laboratory colony were housed in long day (16L: 8D, LD) room after birth. When they turned 35 days old, the animals were transferred to a short day photoperiod (SD: 8L:16D) and fed a standard low fat diet (4.6% calories from fat). After short day acclimation for 70 days, the voles were randomly separated into four groups for another 63 days. The first group remained on the SD photoperiod and continued to be fed on standard low-fat diet (fat content: 4.6% by energy) (SL group: $n = 19$); the second group remained on SD room but were fed on a high-fat diet (fat content: 26.2%) (SH group: $n = 20$); the third group were transferred to a LD photoperiod and fed on the low-fat diet (LL group: $n = 19$); and the fourth group were transferred to LD and fed on the high-fat diet (LH group: $n = 18$). High fat diet (HFD) (22.9 kJ/g, which consisted of 27% fat (soybean oil), 18% protein, 12% crude fiber, and 23% carbohydrate; Beijing HFK Bioscience Co). Low-fat control diet (LFD); 17.5 kJ/g, which consisted of 2.7% fat, 18% protein, 12% crude fiber, and 47% carbohydrate (http://www.hfkbio.com/cms/item/view?table=product&id=90). Body weight was measured daily and food intake was measured at approximately 12 day intervals for 3 days at each time point using metabolic cages (days 0, 12, 24, 36, 48 and 63). We quantified digestible energy intake (DEI), resting metabolic rate (RMR), daily energy expenditure (DEE) and performed glucose tolerance tests (GTT) during the process of photoperiod and food manipulation. After 63 days of treatment all voles were fasted for 3–4 h and sacrificed by CO_2 overdose. The interscapular brown adipose tissue (IBAT), epididymal white fat pad, subcutaneous white fat pad, pancreases, heart, liver, kidneys and testes were immediately and dissected, weighed and stored at −80 °C until assayed. Blood samples were collected, clotted for 1 h and centrifuged at 4 °C for 30 min at 3500 rpm, sera were then collected and stored at −80 °C until assayed.

2.2. Digestible energy intake

Digestible energy intake (DEI) was quantified over the same days as the food intake measurements. Specifically, the remaining food and feces in the metabolic cage after 3 days were collected, oven-dried at 60 °C to a constant mass and separated manually. Dry matter intake (DMI) was calculated from the difference between the food provided and food remaining. The caloric values of food and feces were determined by a Parr1281 oxygen bomb calorimetry (Parr Instrument USA). DEI was then calculated as follows [36]:

DEI (kJ/day) = [Dry matter intake (g/day) × food gross energy (kJ/g)] − [dry faeces mass (g/day) × faeces gross energy (kJ/g)]

Digestibility (%) = DEI (kJ/day)/total energy intake (kJ/day) × 100%

2.3. Metabolic measurements

We measured daily energy expenditure (DEE) using the doubly labelled water (DLW) technique [37] after 27 and 48 days of treatment. Brandt's voles were weighed (±0.1g) and injected with approximately 0.3 g of water containing enriched ^{18}O (31.9 atom %) and ^{2}H (19.0 atom %). Syringes were weighed before and after administration (±0.001 g) to calculate the mass of DLW injected. Blood samples were taken after 1 h of isotope equilibration to estimate initial isotope enrichments [38,39] and were also collected from unlabelled animals to estimate the background isotope enrichments. Blood samples were immediately heat sealed into 2 × 60 µl glass capillaries and stored at room temperature. A final blood sample was taken approximately 48 h later

[38] to estimate isotope elimination rates. Sampling over multiple days minimizes the large day to day variability in DEE [39]. CO_2 production was calculated using the single pool model equation (7.17) [40] as recommended in [41,42] and converted to energy expenditure using the Weir equation [43].

After fifty-five days of treatment resting metabolic rate (RMR) was quantified using indirect calorimetry during the light period (TSE LabMaster, TSE Systems, Germany). Body weight was weighed before each metabolic measurement. RMR was assessed at approximately 30 °C, which is in the thermal neutral zone of Brandt's voles (27.5–32.5 °C [39]). Individually housed Brandt's voles were acclimated to the respirometry chamber and $CO_2 + O_2$ levels were measured every 5 min for 3 h. Animals were not fasted prior to the respirometry run in the chamber. We defined RMR as the average from the 5 min with the least variable and lowest VO_2.

2.4. Intra-peritoneal glucose tolerance test

An intra-peritoneal glucose (2 g/kg body weight) tolerance test was conducted after fasting overnight. Blood samples were taken by tail veni-puncture for glucose measurements by One Touch UltraVue Blood Meter (LifeScan Inc. USA). Immediately before, 15, 30, 60 and 120 min after intra-peritoneal glucose administration, blood glucose was collected and measured. The linear trapezoidal rule was used for estimation of area under the curve (AUC).

2.5. Lipid content of liver, muscle and serum and inflammation markers in serum

Liver and muscle were collected, weighed and oven-dried at 60 °C to constant mass, and then weighed again to obtain the dry mass of tissue. Fat extraction from liver or muscle was performed with a Soxtec Fat Extraction Systems (Soxtec Avanti 2050, FOSS, Sweden), and then fat content was calculated from the ratio between fat mass and dry mass of tissue. Triglyceride and cholesterol contents of liver, muscle and serum were tested using related detection kit (Applygen Technologies Inc., Beijing, China). For the measurement of inflammation markers of TNF-α and IL-6 we utilized mouse ELISA kits (Merck Millipore, USA) on the serum samples according to the supplier's instructions.

2.6. Measurement of UCP1 protein content in eWAT

Total protein in epididymal WAT was lysed in RIPA buffer (1% TritonX-100, 158 mM NaCl, 5 mM EDTA, 10 mM Tris [pH 7.0], protease inhibitor cocktail [Sigma, St. Louis, Missouri, USA], 1 mM DTT, and 0.1% phenylmethylsulfonyl fluoride) Protein concentrations were then determined using the Folin phenol method using bovine serum albumin as the standard. UCP1 content was measured by Western blot in a discontinuous SDS-polyacylamide gel (60 μg/lane, 1c 10.0% running gel and 5% stacking gel). The primary antibodies used were as follows: Rabbit anti-UCP1 diluted 1:5,000 (ab10983, Abcam, Cambridge, MA, USA) and mouse anti-β-tubulin diluted 1:2,000 (E7, DSHB, Iowa City, Iowa, USA) were used as primary antibodies. The secondary antibodies of goat anti-rabbit IgG (1:5,000; ZSGB-BIO Co., Beijing, CHN) and goat anti-mouse IgG (1:5,000; ZSGB-BIO Co., Beijing, CHN) were used respectively against the two primary antibodies.

2.7. Statistical analyses

Data were analyzed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). Group differences among groups in white fat pad distributions, organ masses, DEI, RMR and DEE were analyzed using analysis of covariance (ANCOVA) with body weight as covariate followed by Bonferroni post-hoc tests. Group differences in other parameters (fat free body weight and AUC) were analyzed using analysis of variance (ANOVA). Group differences in body weight daily food intake and gross energy intake, as well as digestible energy intake during acclimation were analyzed using repeated measures ANOVA. Bonferroni correction was used for all pairwise comparisons following significant ANOVA results. Results are presented as means ± SE, and $P < 0.05$ was considered to be statistically significant.

3. RESULTS

3.1. Difference in body weight and body composition among different groups

When voles aged 105 days old were transferred into the long day room, body weight increased and reached maximal levels after 35 days of acclimation (photoperiod: $F_{1,72} = 11.237$, $P < 0.001$, repeated measures ANOVA, Figure 1A). High-fat diet and the interaction of diet and photoperiod had no significant effects on body weight (diet: $F_{1,72} = 0.168$, $P > 0.05$; photoperiod × diet: $F_{1,72} = 0.165$, $P > 0.05$, repeated measures ANOVA, Figure 1A). When exposed to the same photoperiod, high fat diet had no significant effect on average body weight over the last three weeks ($F_{1,73} = 0.313$, $P > 0.05$, Two-way ANOVA, Figure 1B). There was a strong effect of photoperiod during the same period ($F_{1,73} = 11.915$, $P < 0.001$, Two-way ANOVA, Figure 1B) but no significant interaction between diet and photoperiod ($F_{1,72} = 0.099$; $P > 0.05$, Two-way ANOVA, Figure 1B). The sizes of the different organs at the end of the experiment are shown in Table 1. In addition to significant effects on the total body weight and wet carcass mass, photoperiod was also significantly associated with increased epididymal, retroperitoneal and total white fat pad mass (Table 1). High fat diet had no effect on total mass or the wet carcass mass but it was associated with an increase in both retroperitoneal, epidydimal and total fat mass (Table 1). There was no significant interaction between the effects of photoperiod and high fat diet feeding on the weight of any organ. On average the effect of photoperiod on total fat mass was about 80% larger than the effect of HFD. Long day voles had a significantly larger testis and seminal vesicle mass than short day voles. Moreover, iBAT and testis mass of the voles were both decreased by HFD (Table 1).

3.2. High fat diet increased gross energy intake and digestible energy intake of voles but decreased the digestibility

There were significant time effect and interaction between time and diet on food intake over time (time: $F = 26.039$, $P < 0.0001$; time × diet: $F = 7.136$, $P < 0.0001$; repeated measures ANOVA; Figure 2A). High-fat diet, photoperiod and interaction had no significant effect on daily food intake (diet: $F_{1,67} = 2.547$, $P > 0.05$; photoperiod: $F_{1,67} = 0.019$, $P > 0.05$; diet × photoperiod: $F_{1,67} = 0.448$, $P > 0.05$, repeated measures ANOVA; Figure 2A) and cumulative food intake over 63 days (diet × photoperiod: $F_{1,72} = 0.088$, $P > 0.05$; diet: $F_{1,73} = 3.276$, $P > 0.05$; photoperiod: $F_{1,73} = 0.098$, $P > 0.05$, two-way ANOVA; Figure 2B). There was no significant difference in GEI (Gross energy intake) between long day and short day voles, however, voles fed with high-fat diet significantly increased GEI compared with those voles fed with low-fat diet; (diet: $F_{1,67} = 15.523$, $P < 0.0001$; photoperiod: $F_{1,67} = 0.048$, $P > 0.05$; diet × photoperiod: $F_{1,67} = 0.486$, $P > 0.05$; repeated measures ANOVA, Fig 2C). The long day photoperiod and short day photoperiod had no significant effect on gross energy intake over 63 days ($P > 0.05$; Figure 2D). Voles fed with high-fat diet had significantly higher DEI (Digestible energy intake) than those voles fed with low-fat diet; however, there was no significant

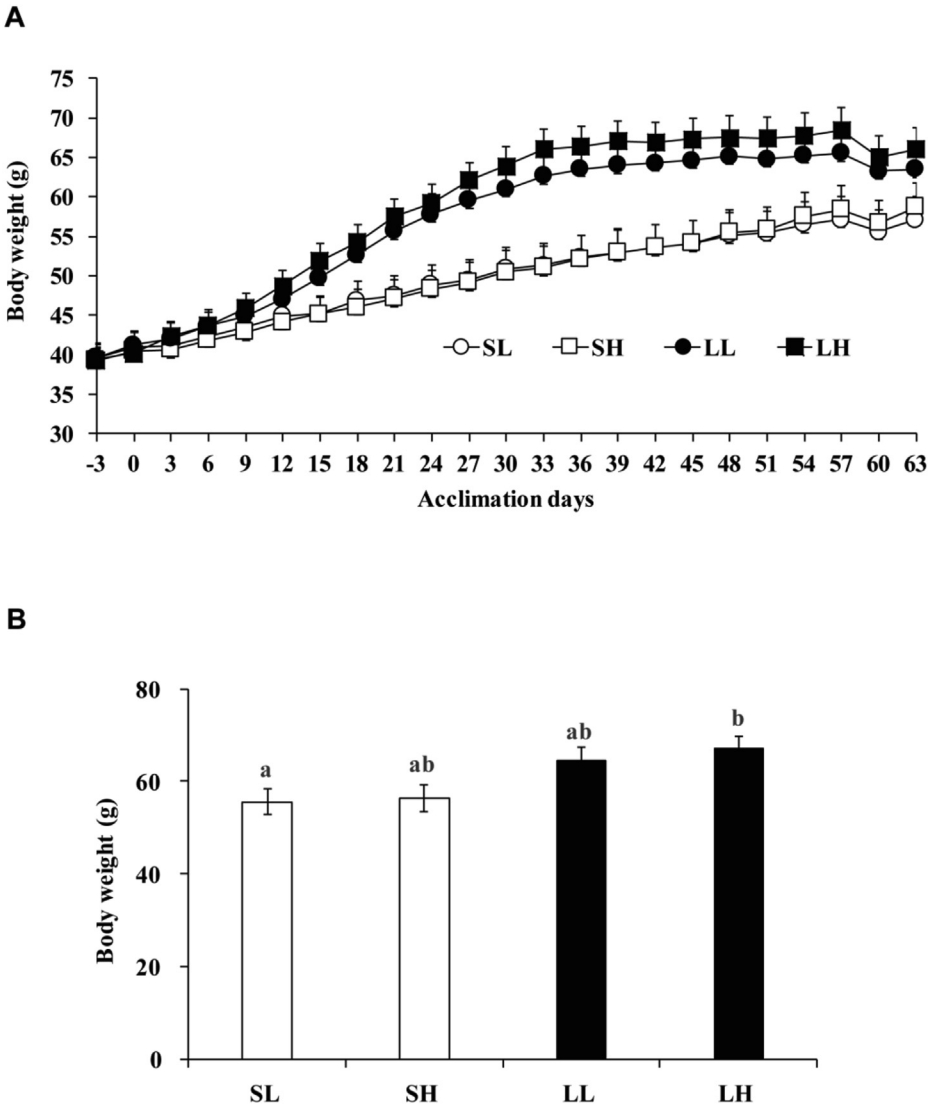

Figure 1: Effects of photoperiod and food treatment on body mass of the voles. Seventy-six voles were exposed to short day photoperiod with low fat diet (fat content: 4.6%; LFD) and then 19 of them switched to a long day photoperiod (LD + LFD: LL) on day0, while 20 of them were fed with high fat diet (fat content: 26.2%; HFD) continue under short day photoperiod (SD + HFD: SH), and 18 of them switched to a long day photoperiod and fed with high fat diet (LD + HFD: LH), as well as the remainder (n = 19) not changed (SD + LFD: SL). (A) Body weight of SL, SH, LL and LH over the 63 days of treatments. (B) Average body weight of the last three weeks of measurement. a and b represent $p < 0.05$. Groups with at least one same letter were not significantly different. Values are means ± SE (n = 18–20 of each group).

difference between long day and short day voles (diet: $F_{1,67} = 7.314$, $P < 0.01$; photoperiod: $F_{1,67} = 0.001$, $P > 0.05$; diet × photoperiod: $F_{1,67} = 0.490$, $P > 0.05$; repeated measures ANOVA, Figure 2E). Digestibility was decreased in voles fed with high-fat diet compared voles fed with low-fat diet; however, photoperiod had no effect on digestibility (diet: $F_{1,67} = 103.881$, $P < 0.001$; photoperiod: $F_{1,67} = 2.724$, $P > 0.05$; diet × photoperiod: $F_{1,67} = 0.347$, $P > 0.05$; repeated measures ANOVA, Figure 2F).

3.3. High fat diet impaired the glucose tolerance independent of body weight

After 56 days of treatment, the IPGTT indicated that there was no significant difference in blood glucose concentrations between long day and short day animals (Figure 3A). Glucose area under the curve (AUC) from 0 to 120 min showed no difference between long day and short day animals (Figure 3B). In contrast, glucose tolerance was impaired by high-fat diet compared with controls as indicated both by change curve and area under curve (diet: $F_{1,71} = 24.697$, $P < 0.001$; photoperiod: $F_{1,71} = 0.006$, $P > 0.05$; diet × photoperiod: $F_{1,71} = 4.674$, $P < 0.05$; Two-way ANOVA; Figure 3A,B). No significant correlation was found between AUC and body weight ($P > 0.05$; Figure 3C), as well as body fat mass ($P > 0.05$; Figure 3E). Body weight had significant effect on AUC ($F_{1,71} = 4.015$, $P < 0.05$; ANCOVA). High fat diet-fed voles exhibited slower glucose clearance compared to voles fed with low fat diet when corrected by body weight ($P < 0.0001$, Two-way ANOVA; Figure 3D) and body fat mass ($P < 0.0001$, Two-way ANOVA; Figure 3F). But meanwhile, the long day and short day photoperiod had no significant effect on the area under curve both adjusted by body weight and body fat mass ($P > 0.05$, Two-way ANOVA; Figure 3D,F).

Table 1 — Body composition of different groups of Brandt's voles exposed to photoperiod and diet manipulations. The statistics show the raw p values in two-way ANOVA analysis and those highlighted in bold were significant. The direction of the effect is also indicated. RWAT = (insert details of all the abbreviations).

	Group				Statistics		
Parameters	SL ($n = 19$)	SH ($n = 20$)	LL ($n = 19$)	LH ($n = 18$)	Photoperiod	High-fat diet	Interaction
Final body mass(g)	55.6 ± 3.0	57.7 ± 3.0	61.5 ± 3.1	64.6 ± 2.6	**0.035 (+)**	0.386	0.858
Wet carcass (g)	37.0 ± 2.2	37.9 ± 2.0	39.8 ± 1.8	40.2 ± 1.3	**0.006(+)**	0.068	0.173
RWAT (mg)	694 ± 93	837 ± 107	1047 ± 124	1415 ± 140	**0.001(+)**	**0.037(+)**	0.206
EWAT (mg)	984 ± 135	1155 ± 130	1175 ± 136	1838 ± 218	**0.012(+)**	**0.003(+)**	0.083
Total WAT (mg)	5.1 ± 0.5	6.0 ± 0.6	7.2 ± 0.8	9.4 ± 0.9	**0.002(+)**	**0.043(+)**	0.254
IBAT (mg)	287 ± 29	230 ± 21	309 ± 28	295 ± 31	0.991	**0.007(−)**	0.278
Testis (mg)	881 ± 66	792 ± 58	1073 ± 40	989 ± 33	**0.001(+)**	**0.001(−)**	0.940
Seminal vesicles (g)	630 ± 75	509 ± 66	782 ± 57	804 ± 53	**0.005(+)**	0.127	0.224
Brain (mg)	578 ± 8	583 ± 10	568 ± 8	690 ± 11	0.457	0.253	0.325
Heart (mg)	242 ± 12	357 ± 11	257 ± 10	255 ± 9	0.182	0.415	0.260
Liver (mg)	1805 ± 88	1990 ± 179	2066 ± 187	2394 ± 162	0.367	0.270	0.441
Spleen (mg)	33 ± 2	39 ± 4	33 ± 2	35 ± 3	0.087	0.141	0.344
Kidneys (mg)	599 ± 29	550 ± 27	647 ± 32	577 ± 23	0.383	**<0.001(−)**	0.326
Pancreas (mg)	57 ± 3	52 ± 3	52 ± 3	54 ± 5	0.085	0.270	0.298

3.4. Voles fed with high fat diet had a lower daily energy expenditure at 4 weeks compared with voles fed with low fat diet

Both photoperiod and high-fat diet had no effect on RMR in voles at day 55 days (diet: $F_{1,72} = 0.137$, $P > 0.05$; photoperiod: $F_{1,72} = 1.309$, $P > 0.05$; diet × photoperiod: $F_{1,72} = 0.199$, $P > 0.05$; Two-way ANOVA, Figure 4A). Analysis of covariance (ANCOVA) with body weight as a covariate showed that there was no significant difference in RMR among groups when corrected for body weight ($P > 0.05$; Figure 4B,C). Photoperiod had no effect on DEE in voles at both time points and high fat diet decreased DEE at week 4 but not week 8 after adjustment by body weight (WEEK 8: diet: $F_{1,75} = 0.809$, $P > 0.05$; photoperiod: $F_{1,75} = 0.013$, $P > 0.05$; diet × photoperiod: $F_{1,75} = 2.295$, $P > 0.05$; Two-way ANOVA, Figure 4G-I; WEEK 4: diet: $F_{1,57} = 5.362$, $P < 0.05$; photoperiod: $F_{1,57} = 0.006$, $P > 0.05$; diet × photoperiod: $F_{1,57} = 0.070$, $P > 0.05$; Two-way ANOVA, Figure 4D-F). The direct measures of metabolic rate were only available at one time point (around day 56). We reconstructed the changes in metabolic rate throughout the experiment from the patterns of change in DEI and changes in body weight (assuming that all the weight change was fat with an energy density of 39 kJ/g). There was a significant time effect, and interaction between time and photoperiod effect, on change in body weight over time (time: $F = 59.1$, $P < 0.0001$; time x photoperiod: $F = 29.9$, $P < 0.0001$; GLM-repeated measures ANOVA; Figure 4J). The 2-way interaction between time and diet and the 3-way interaction of time, photoperiod and diet both had no significant effect on change in body weight (time x diet: $F = 0.977$, $P > 0.05$; time x photoperiod x diet: $F = 0.75$, $P > 0.05$; GLM-repeated measures ANOVA; Figure 4J). As for the reconstructed metabolic rate, there was a significant time effect and significant interaction between time and photoperiod effect (time: $F = 199.1$, $P < 0.0001$; time x photoperiod: $F = 10.430$, $P < 0.0001$; GLM-repeated measures ANOVA; Figure 4K), while the 2-way interaction between time and diet and the 3-way interaction between time, photoperiod and diet both had no significant effect on metabolic rate (time x diet: $F = 2.265$, $P > 0.05$; time x photoperiod x diet: $F = 1.480$, $P > 0.05$; GLM-repeated measures ANOVA; Figure 4K). Voles exposed to long photoperiods showed a transient decrease in their metabolic rates around day 24 coincident with the time when they were gaining most weight (Figure 4J,K).

3.5. Fat content of liver and muscle and inflammation markers in serum

After 63 days acclimation, high fat diet increased fat content of muscle (diet: $F_{1,70} = 6.751$, $P < 0.05$; photoperiod: $F_{1,70} = 1.360$, $P > 0.05$; diet × photoperiod: $F_{1,70} = 0.395$, $P > 0.05$; Figure 5C,D) but decreased fat content of liver (diet: $F_{1,71} = 4.895$, $P < 0.05$; photoperiod: $F_{1,71} = 0.013$, $P > 0.05$; diet × photoperiod effect: $F_{1,71} = 0.391$, $P > 0.05$; Two-way ANOVA; Figure 5A,B). In contrast, photoperiod had no effect on fat content both in muscle and liver (Figure 5A,C). Long day treatment increased triglyceride content of serum ($P < 0.05$, ANOVA; Table 2). High fat diet increased cholesterol content of liver, muscle and serum ($P < 0.05$, ANOVA; Table 2) but decreased triglyceride content of liver ($P < 0.05$, ANOVA, Table 2).
Both high-fat diet and photoperiod had no effect on the TNF-α level in serum (diet: $F_{1,35} = 0.311$, $P > 0.05$; photoperiod: $F_{1,35} = 0.054$, $P > 0.05$; diet × photoperiod: $F_{1,35} = 2.673$, $P > 0.05$; Two-way ANOVA, Figure 5D). IL-6 level in serum was increased by high-fat diet specifically in short day voles (diet: $F_{1,35} = 5.040$, $P < 0.05$, Two-way ANOVA, Figure 5E); however, it was not affected by photoperiod (photoperiod: $F_{1,35} = 1.265$, $P > 0.05$; diet × photoperiod: $F_{1,35} = 6.288$, $P < 0.05$, Two-way ANOVA, Figure 5E).

3.6. Effect of photoperiod and high fat diet on UCP1 protein levels in epididymal WAT

UCP1 expression in epididymal WAT of voles was decreased in voles housed in LD photoperiod compared with those on SD ($P < 0.05$, one-way ANOVA, Figure 6A); but high fat diet had no effect on UCP1 protein expression (diet: $P > 0.05$; photoperiod: $P < 0.05$; diet × photoperiod: $P > 0.05$, Two-way ANOVA, Figure 6A). A negative relationship was observed between UCP1 protein levels in epididymal WAT and body weight (Figure 6B) and body fat mass (Figure 6C). However, both photoperiod and high fat diet had no significant effect on UCP1 expression in BAT (Figure 6D).

4. DISSCUSSION

Consistent with our previous studies of voles (bank, Brandts', and short-tailed field voles) there was a significant positive effect of long day photoperiod exposure on the total body weight and body fat content of the Brandt's vole [29,31–33]. We have previously shown in

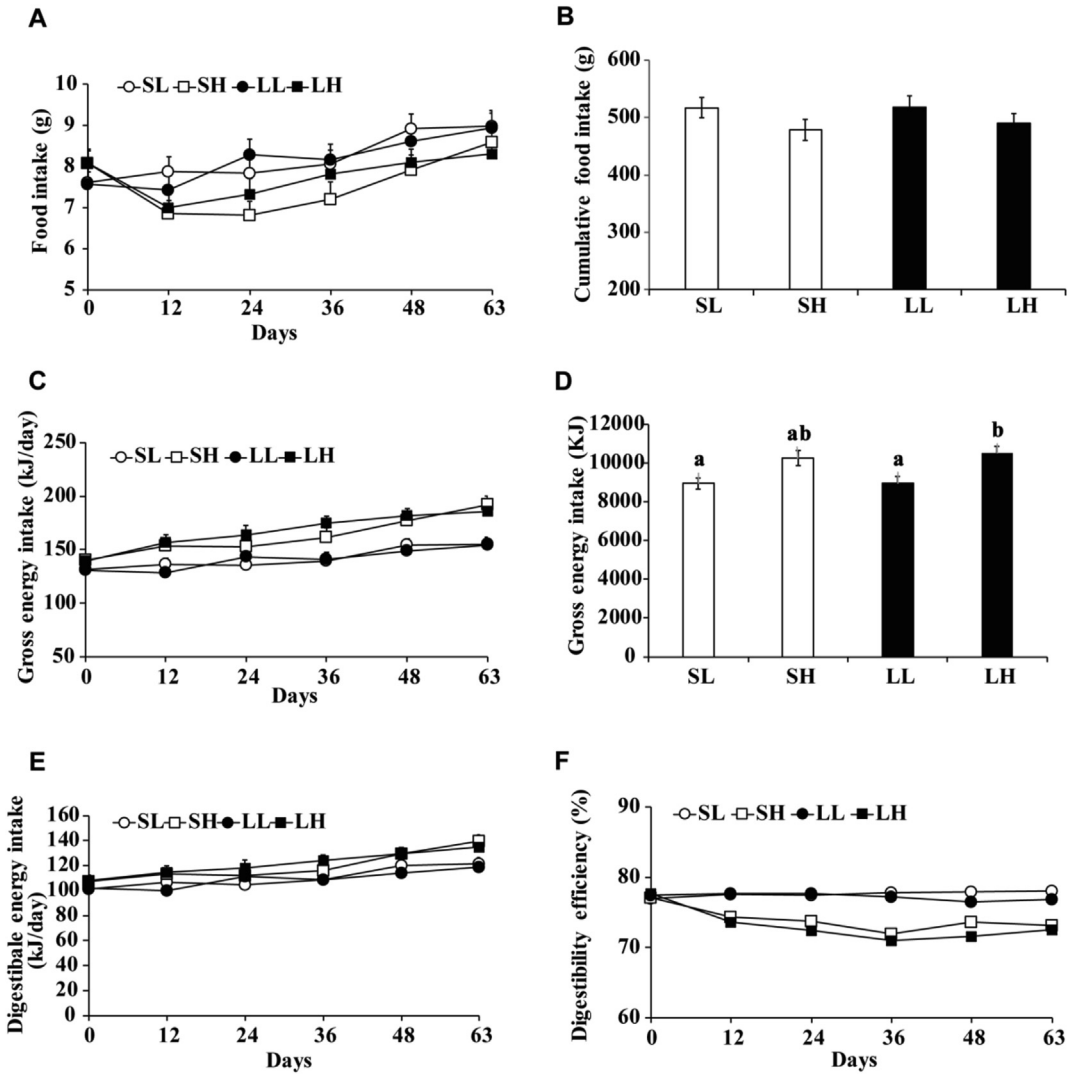

Figure 2: Effects of photoperiod and food treatment on food intake, gross energy intake and digestible energy intake of Brandt's voles. (A) Food intake of voles changed over time. (B) Cumulative food intake of voles over 63 days. (C) Gross energy intake per day changed over time. (D) Gross energy intake of voles over 63 days. (E) digestible energy intake calculated as energy in the food minus energy excreted in faeces, and (F) digestive efficiency-the percent of ingested food that is absorbed. a and b represent $p < 0.05$. Groups with at least one same letter were not significantly different. Values are means ± SE (n = 18–20 of each group).

bank voles that under SD photoperiod they are resistant to fat gain when fed a HFD [32]. In contrast to the effect of HFD under short photoperiod in bank voles, we found here that while HFD had no effect on total body weight there was a significant effect on the size of individual fat stores and also on the total fat mass (Table 1). The magnitude of this HFD effect on total fat mass was 0.9g under SD and 2.2g under LD (equal to 1.6–3.5% of total body weight). This apparently slightly larger effect in the LD condition was consistent with the previous work [34], although in our case the interaction effect of photoperiod and diet was not significant, suggesting there was no difference in the effect of HFD under different photoperiods.

Although there was a statistically significant effect of HFD on adiposity, this impact was considerably smaller than is typically observed after 9 weeks of high fat feeding in DIO mouse models. Direct comparisons with the literature however are difficult because most studies of DIO in mice use the commercially available diets (e.g. from research diets D12451 or D12492) that have 45–60% fat content (by energy), compared to the diet used here which had 26% fat. Brandts' voles would not eat the higher fat DIO mouse diets. However, C57BL/6 mice exposed to a 25% fat diet for 9 weeks increased their fat mass from 4.3 to 7.6 g (a 3.3g increase from a starting weight of 30.1g giving an increase equal to 11% of body weight) [10]. This effect was 3 to 7x greater than the relative increase in fat mass of the Brandt's vole reported here, on a similar dietary fat level for the same duration, suggesting that compared to mice the Brandt's vole is indeed resistant to fat deposition on a HFD. In bank voles, the animals showed no increase in total or fat mass when fed a diet containing 28.2% fat [32] suggesting the difference between the vole species was not due the dietary fat level they were exposed to.

Previous work suggested that increased adiposity in voles fed with HFD was mainly due to the increased energy intake and not reduced energy expenditure [44–48]. Our study supported these previous observations. We found the cumulative digested energy intake for 63 days of high-fat diet fed Brandt's voles was higher than that of low-fat diet group in both long photoperiod (+11.64%) and short photoperiod (+8.43%). The reconstructed patterns of energy expenditure through

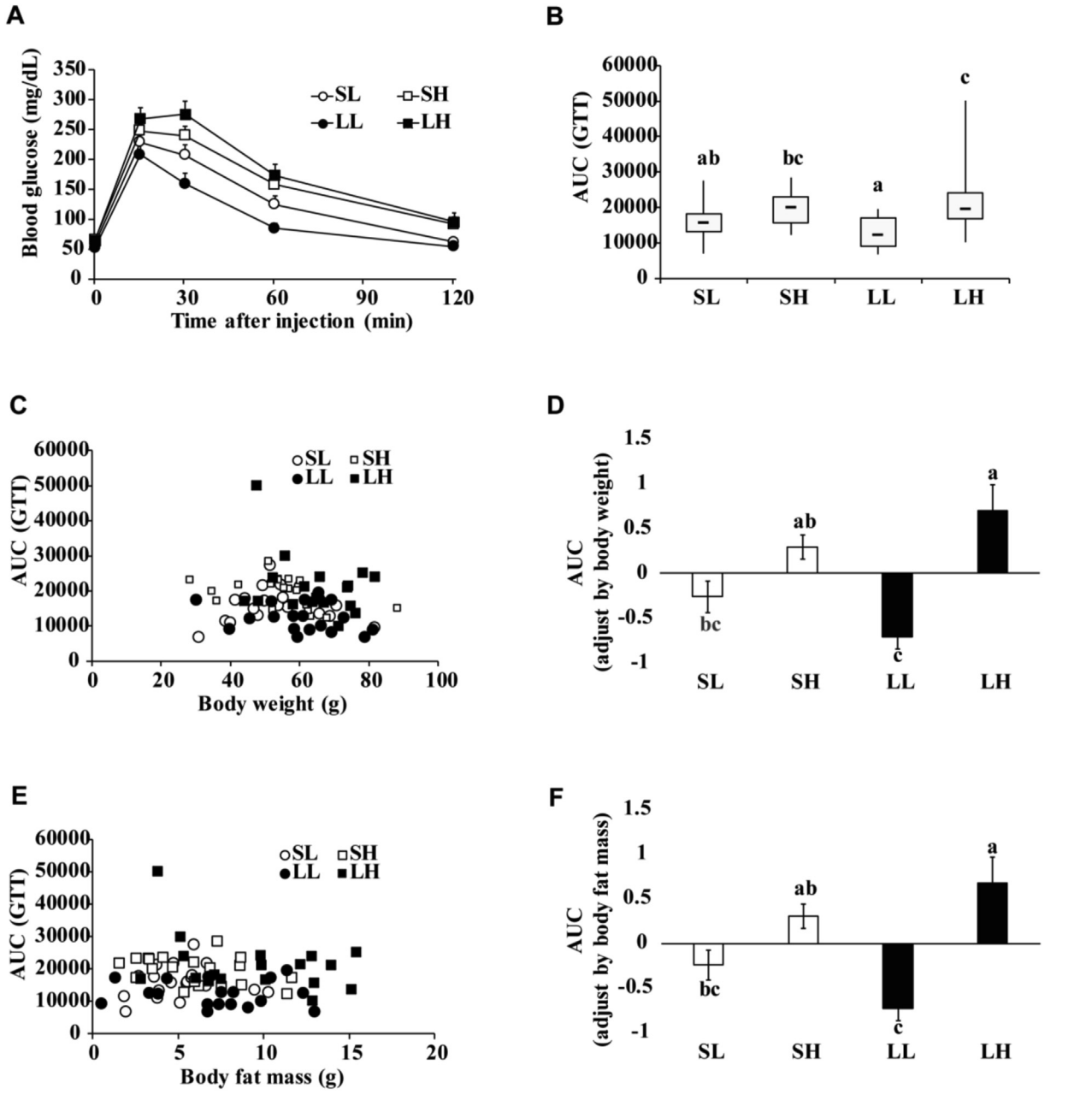

Figure 3: Effects of photoperiod and food treatment on glucose tolerance of the voles. (A) Blood glucose concentrations after 0, 15, 30, 60 and 120min of glucose injection in each group. (B) Area under curve (AUC) in (A). (C and E) Regression between AUC and body weight (C) and body fat mass (E). (D and F) AUC adjusted by body weight (D) and by body fat mass (F). a, b and c represent $p < 0.05$. Groups with at least one same letter were not significantly different. Values are means ± SE ($n = 18–20$ of each group).

time, showed that during the period when the long photoperiod voles were accumulating body weight (Figure 4J) at day 24 there was also a transient reduction in metabolism (Figure 4K). This is consistent with our previous work where we also detected a transient decrease in basal metabolic rate during this period [29]. Different species seem to enable to photoperiod induced obesity in different ways. In short-tailed field voles the greater adiposity under long photoperiod stemmed mostly from increased digestibility [25,26]. This contrasts the effect in Lemmings (*Dicrostonyx groenlandicus)* where modulated expenditure is an important factor in weight and fat accumulation under altered photoperiod [49,50] as suggested here also for the Brant's vole. The reconstructed time course of metabolic rate was also consistent with the direct measures of metabolism (Figure 4A–I) which showed no difference between groups at days 48–63. This suggests there were no lasting metabolic rate differences due to either photoperiod or high fat diet feeding.

The photoperiod effect on adiposity was about 8× larger than the diet effect. This difference in response allows us to partition the effects of obesity and diet on glucose homeostasis. Consistent with our previous work [29] we demonstrated that photoperiod-induced obesity did not result in glucose intolerance. This absence of an impact of the photoperiod-induced obesity on glucose homeostasis was paralleled by an absence of any increase in the fat content of the liver and skeletal muscle. In contrast there was a strong negative effect of the high fat

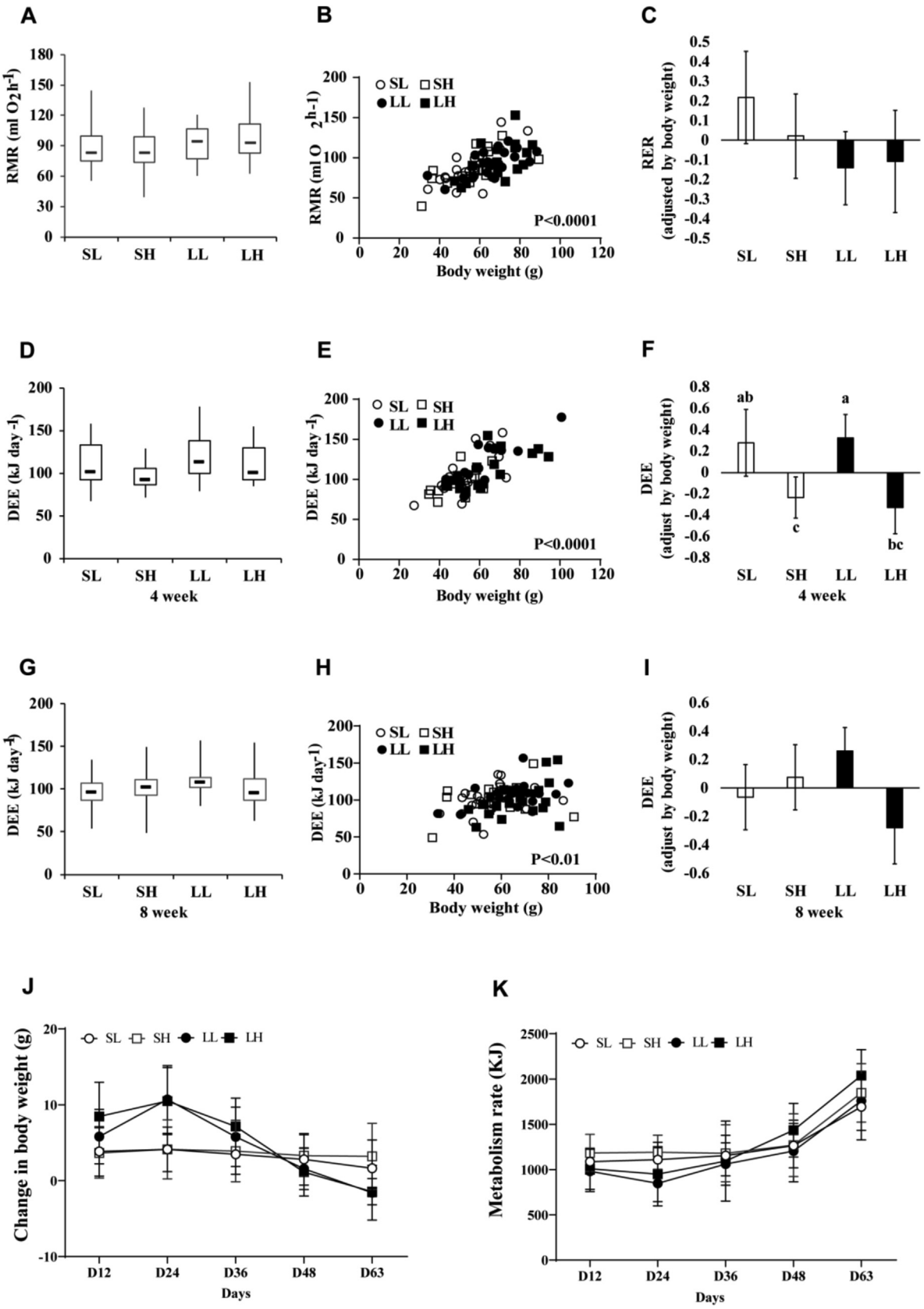

Figure 4: Effects of photoperiod and food treatment on resting metabolic rate (RMR) of the voles (A) and daily energy expenditure (DEE) of the voles at 8 (D) and 4 (G) weeks of treatment. Regression between body weight and RMR (B), DEE of 4 weeks (E) and DEE of 8 weeks (H). RMR (C), DEE of 4 weeks (F) and DEE of 8 weeks (I) adjusted by body weight. a, b and c represent $p < 0.05$. Change in body weight (J) and metabolism rate of voles over time (K). Groups with at least one same letter were not significantly different. Values are means ± SE (n = 18–20 of each group).

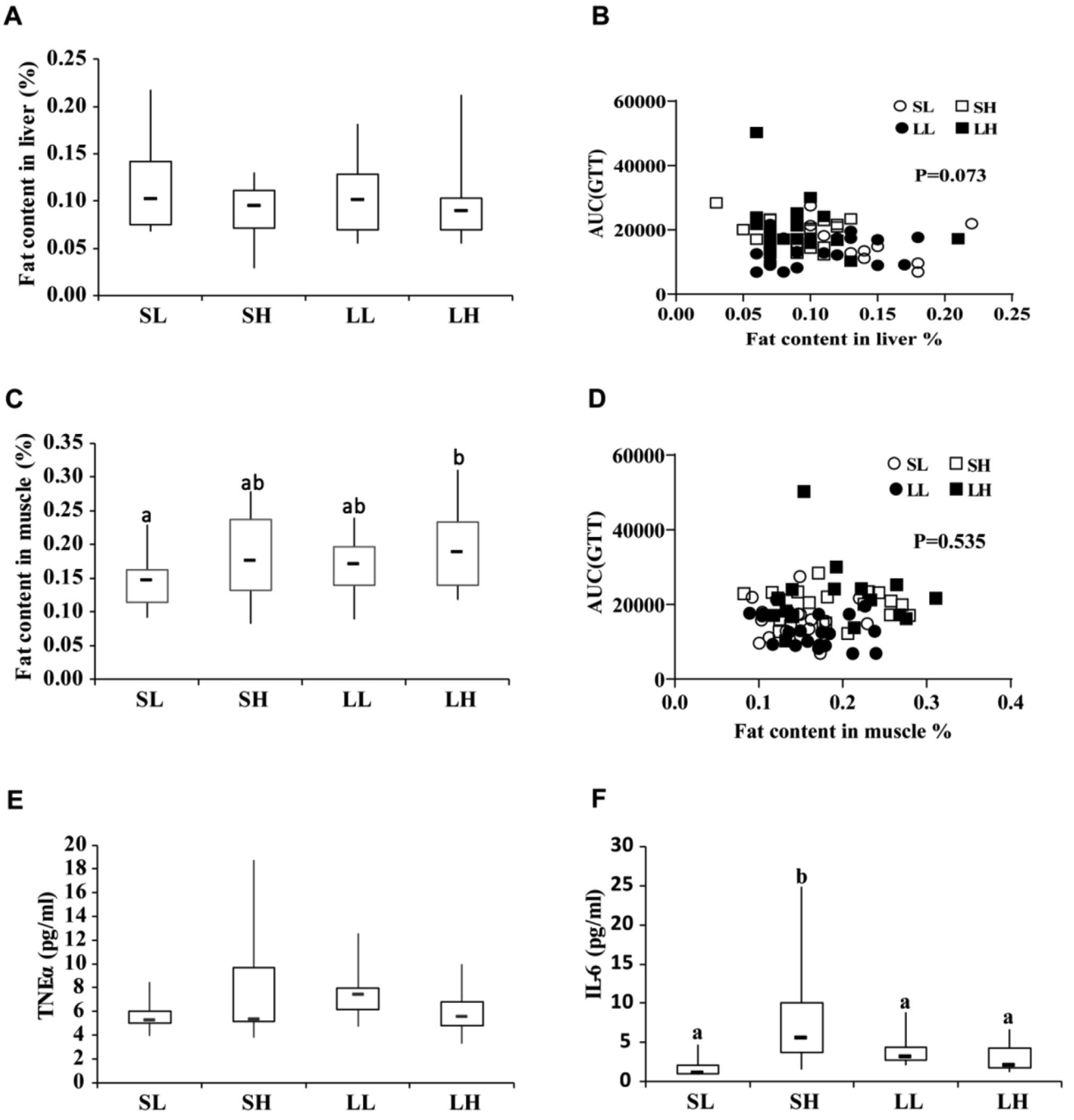

Figure 5: Effects of photoperiod and food treatment on liver and muscle fat content, serum TNF-α and IL-6 of the voles. (A and C) The percentage of fat content in liver (A) and in muscle (C) (B and D) Regression between AUC and the percentage of fat content in liver (B) and in muscle (D). (E and F) Serum TNF-α (E) and IL-6 (F) of the voles. a and b represent $p < 0.05$. Groups with at least one same letter were not significantly different. Values are means ± SE ($n = 18-20$ of each group).

diet manipulation on glucose homeostasis. This was accompanied by ectopic fat deposition in muscle, but surprisingly not in liver. Our data are thus consistent with the idea that ectopic fat deposition in key metabolic organs is a key factor leading to insulin resistance [51—57].

Previously in mice we showed that glucose homeostasis was strongly correlated with body fatness, and once that effect was accounted for there was no further impact of diet [11]. In other words, diet effects were only mediated via adiposity. This result seemingly conflicts with the findings here that diet had much greater impact than adiposity. The differing effects of different causes of fat accumulation on ectopic fat deposition however potentially resolve this conundrum [47,58]. In mice expanding adiposity may be linked to additional fat deposition in the liver and muscle causing the impaired glucose homeostasis in the same way that expanding fat tissue in the voles that resulted form the HFD was also linked to such effects. However, expanding fat due to the photoperiod change was not linked to such effects on ectopic fat and had no impact on glucose homeostasis. Over the course of evolution

Table 2 — Triglyceride and cholesterol contents of liver, muscle and serum among different groups in Brandt's voles.

Parameters	Group				Statistics		
	SL ($n = 19$)	SH ($n = 20$)	LL ($n = 19$)	LH ($n = 18$)	Photoperiod	High-fat diet	Interaction
Triglyceride in serum (mmol/L)	0.733 ± 0.100	0.680 ± 0.097	1.078 ± 0.100	1.106 ± 0.103	**<0.001 (+)**	0.902	0.687
Triglyceride in liver (mmol/g protein)	0.142 ± 0.012	0.081 ± 0.012	0.132 ± 0.012	0.074 ± 0.013	0.488	**<0.001 (−)**	0.889
Triglyceride in muscle (mmol/g protein)	0.151 ± 0.017	0.164 ± 0.017	0.174 ± 0.017	0.140 ± 0.017	0.996	0.529	0.175
Cholesterol in serum (mmol/L)	0.259 ± 0.010	0.281 ± 0.010	0.226 ± 0.010	0.299 ± 0.010	0.450	**<0.001 (+)**	**0.012**
Cholesterol in liver (mmol/g protein)	0.082 ± 0.004	0.098 ± 0.004	0.080 ± 0.004	0.101 ± 0.004	0.927	**<0.001 (+)**	0.450
Cholesterol in muscle (mmol/g protein)	0.041 ± 0.005	0.086 ± 0.005	0.040 ± 0.005	0.093 ± 0.006	0.601	**<0.001 (+)**	0.468

voles have probably evolved mechanisms to deal with the potentially negative consequences of seasonally expanding fat tissue, but they have not evolved to deal with the consequences of expanding fat due to a (high fat) diet that they never encounter in the wild. The same is probably also true for mice exposed to high fat diets that drive adiposity. At present two issues remain unclear and will be a focus for future work. How do voles exposed to long photoperiods and massively expanding fat stores avoid ectopic fat deposition, and why was the fat deposition only increased in muscle, but not in liver, in voles fed with high-fat diet.

A limitation of this work is that we did not present any measurements of insulin levels or insulin sensitivity. In fact we did measure these things and the data are presented in Supplementary Fig. 1. Since we did not find the expected elevation of insulin levels in relation to body fatness, nor any significant impact on insulin sensitivity we were concerned that the Rat ELISA assay we used to assay insulin levels did not show cross species reactivity, and these data are therefore not reflective of actual insulin levels in the voles. Similarly, the absence of any effect on insulin sensitivity may relate to issues with voles responding to human insulin. At the moment the genome of the Brandt's vole has not been sequenced so we are unable to check homology of the insulin gene or its receptor. Understanding insulin changes in these animals is a future goal of our work.

In summary, exposing voles to photoperiod and HFD allowed us to separate the impacts of diet and adiposity on glucose homeostasis. There was a large effect of HFD feeding on glucose intolerance but no effect of adiposity that stemmed from the photoperiod change. This impairment of glucose homeostasis under HFD feeding was accompanied with changed ectopic fat deposition particularly in skeletal muscle. The same impacts on both glucose homeostasis and ectopic fat deposition were not associated with expanding adiposity driven by photoperiod change. This suggests that impairment of glucose homeostasis is largely driven by the level of any particular manipulation on ectopic fat storage. If the same is true in humans this may explain

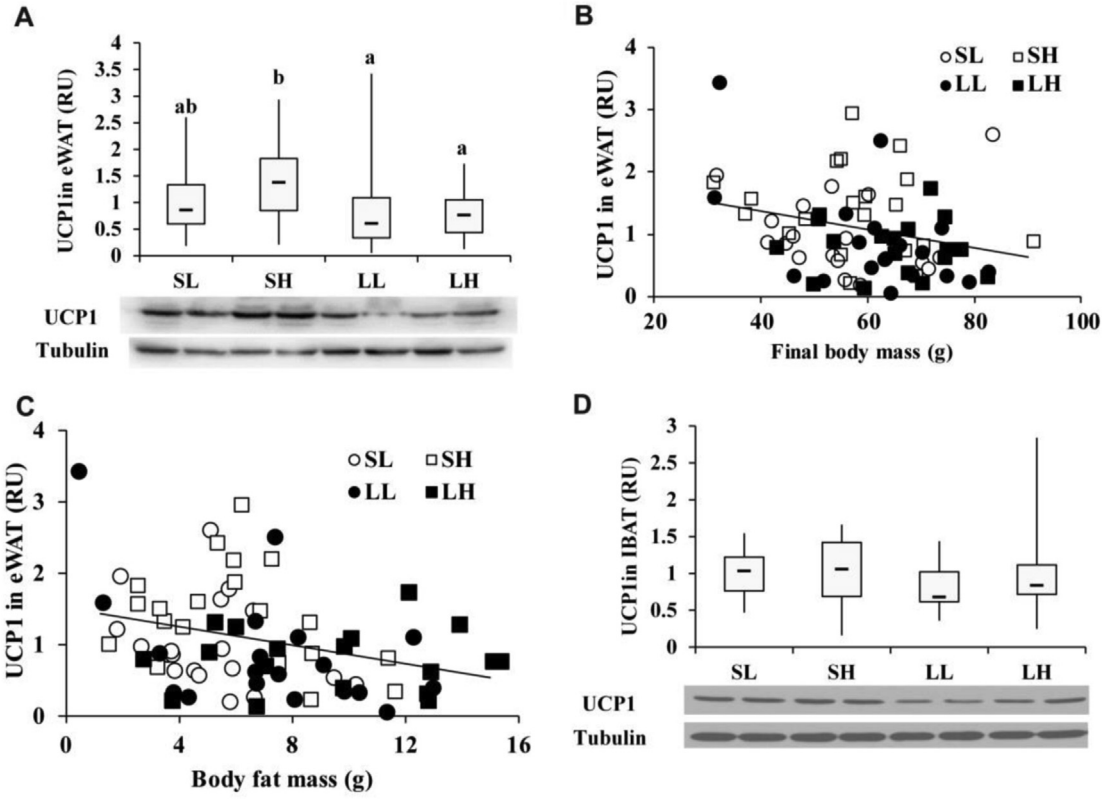

Figure 6: UCP1 expression in epididymal WAT (A) and BAT (D) of Brandt's voles after photoperiod and high fat diet treatment and its relationship with final body mass (B) and body fat mass (C). Groups with at least one same letter were not significantly different. Values are means ± SE (n = 18–20 of each group).

why some people are able to have obesity yet avoid the metabolic dysfunction that is associated with it.

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DATA AVAILABILITY

Data will be made available on request.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molmet.2023.101724.

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